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(54) Title: VACCINES FROM TAXONOMICALLY SIMILAR ORGANISMS

(57) Abstract

A vaccine for protection against an infectious organism which cannot be readily cultured in vitro comprising an antigen derived from a taxonomically similar organism which is readily cultured in vitro. The method of preparation and the method of use are also disclosed.

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Description

VACCINES FROM TAXONOMICALLY SIMILAR ORGANISMS

Technical Field

This invention is directed to vaccines and their preparation from organisms taxonomically similar to the infective organism.

Background Art

Immunotherapy with respect to infectious organisms has generally followed the theory of using either the organism itself or some intermediate state in its development as the basis for production of an immunizing vaccine. In this regard, the art is replete with examples of the effectiveness of this approach. For instance, immunization of chickens against cecal coccidiosis by establishing a controlled subclinical infection (U.S. Patent Number 3,147,185); non-living vaccine produced by incubation of third-stage nematode larvae into the histotrophic stages (U.S. Patent Number 3,395,218 and German Auslegesschrift 1,160,139); canine hookworm vaccine comprising a physiologically acceptable aqueous vehicle containing attenuated premigratory live hookworm larvae (U.S. Patent Number 3,657,415); an Ascaris suum vaccine comprising sonicated third-stage larvae, sonicated second stage larvae, larvae hatching fluid and second-stage larval culture fluid (U.S. Patent Number 3,676,547); an antigen preparation for immunoprecipitin diagnostic testing for Chagas' disease, caused by Trypanosoma cruzi, comprising purified, water-soluble antigen obtained from tissue culture of Trypanosoma cruzi

to essentially only the trypomastigote and amastigote growth stages(U.S. Patent Number 3,911,097); vaccine comprising a live, but attenuated, metazoan endoparasite which is pathogenic to domestic animals and is a nematode, trematode or cestode in the form of eggs or a premigratory or a migratory immature form (British Patent Number 819,830 and Canadian Patent Number 602,465); a vaccine comprising a metazoan or protozoan endoparasite, which elicits an immune response in the host, attenuated with a sub-lethal dose of ultraviolet radiation (British Patent Number 902,760) and a vaccine comprising an antigen of Schistosoma mansoni separated from a development stage of the parasite (German Offenlegungsschrift 2,742,835). Additionally, a vaccine for canine hookworm has been produced which comprises a physiologically acceptable aqueous vehicle containing premigratory live hookworm larvae of a hookworm species which is specific to cats (British Patent Number 1,277,134).

However, a need continues to exist for the preparation of vaccines against infectious organisms, which are either difficult or impossible to culture, in vitro, on a commercially practical scale. Exemplary of infectious organisms which cannot be readily cultured, in vitro, on a commercial scale are Ichthyophthirius multifiliis and Cryptocaryon irritans.

Ichthyophthirius multifilius and Cryptocaryon irritans are some of the most damaging parasites of warm water and salt water fish, respectively, and some of the most difficult to control. Experimental immunization of catfish with Ichthyophthirius multifiliis has been reported (Becker et al,"Some Host Response of White Catfish to Ichthyophthirius multifiliis Fouquet", Proc. 18th Ann. Conf. S.E. Assoc. Game and Fish Comm., 1964). Additional studies have shown that channel catfish injected intraperitoneally with vaccine prepared from the ground tro-

phozoites, with and without Freund's adjuvant, survived challenge whereas controls suffered 100% mortality after seven days (Areerat, S., "The Immume Response of Channel Catfish, Ictalurus punctatus (Rafinesque) to Ichthyophthirius multifiliis", Masters Thesis, Auburn University, Auburn, Alabama (1974)).

No known method exists for growing the protozoans Ichthyophthirius multifiliis and Cryptocaryon irritans, in vitro. Both Ichthyophthirius multifiliis and Cryptocaryon irritans are obligate parasites, the infective tomites must penetrate a host within 24-48 hours or perish. For this reason, maintenance of the parasites requires continual passage to susceptible hosts. Therefor, the accumulation of sufficient antigen to immunize large numbers of fish is very time-consuming and impractical.

Ichthyophthirius multifiliis and Cryptocaryon irritans are devastating parasites because they have a high morbidity and mortality. The diseases affect not only food fish, including trout, salmon, catfish, carp, eel, tuna, and bonita, but also ornamental fish. At present, chemical treatments are the only practical way of controlling the disease. These treatments including malachite green, formalin, methylene blue, potassium permanganate and others are not approved for use on food fish by the USDA.

Furthermore, malachite green, the most effective treatment, may soon be banned from use on any fish. Because of the limitations of chemotherapy, immunotherapy may be the only successful and practical approach to controlling these diseases.

Disclosure of the Invention

Accordingly, one object of the invention is to provide an effective vaccine for immunization against infectious organisms which are difficult or impossible to culture, in vitro

A further object of the invention is to provide a vaccine for immunization against a pathogen derived from a taxonomically similar organism.

A further object of the invention is to provide a commercially feasible method of producing a vaccine for immunization against infectious organisms on a scale suitable for commercial operations.

A further object of the invention is to provide an effective vaccine for the immunization of fish against Ichthyophthirius multifiliis and Cryptocaryon irritans.

A further object of the invention is to provide a commercially feasible method of producing a vaccine for fish on a scale suitable for aquacultural operations.

Briefly, these objects and other objects of the invention as hereinafter will become more readily apparent can be obtained by providing a vaccine for protection against infection by an organism which cannot be readily cultured in vitro which comprises an antigen derived from a taxonomicaly similar organism which may be readily cultured, in vitro.

Best Mode for Carrying Out the Invention

Taxonomy is the orderly classification of organisms into appropriate categories on the basis of relationships among them, e.g. species, genus, family, order or class. Applicants have discovered that by careful analysis of these relationships, it is possible to select suitable pairs of organisms wherein antigenic cross-reactivity may be used to develop a vaccine for one of

the organisms based on the other of the organisms.

Taxonomic similarity is now found to be an ideal basis for selection of organisms suitable for use in the preparation of a vaccine. Taxonomic similarity is determined through fluorescent staining techniques. In particular, an animal, such as a rabbit, is innoculated with the pathogen; blood serum is collected from the rabbit, i.e. antibodies are collected; the pathogen is then contacted with the blood serum, allowing interaction of the antigen and antibody; the so-contacted pathogen is then stained with a fluorescent stain which is selective for the site of antibody-antigen interactin, e.g. staining with FITC labelled anti-rabbit IgG; the gross morphology of the so-stained pathogen is then examined to determine the sites of greatest intensity of staining, e.g. cilia, flagella, mouth and the like; based on the localization of high intensity staining, an organism, suitable for use as a source of vaccine, is selected which has identical physical features to those producing the highest intensity of staining in the pathogen. Desirability, additional factors such as body shape, pathogenicity for the host and ease of culturation may be used in selection of the organism suitable for preparation of a vaccine. For example, the minimum taxonomic relationship appropriate as an indicia of antigenic cross-reactivity for the parasites Ichthyophthirius multifiliis and Cryptocaryon irritans would be that of a ciliate. In particular, for the protozoans Ichthyophtirius multifiliis and Cryptocaryon irritans, a protozoan having the characteristics of being easily cultivable, having cilia and being pathogenic for fish would be highly suitable. Such ciliate organisms would induce immunity, to Ichthyophthirius multifiliis and Cryptocaryon irritans, when used in the preparation of vaccine.

This approach allows the selection of a particular

organism, to be cultured for the production of antigen, wherein the organism selected may be selected on the basis of commercial viability - i.e. ease of production and cost, rather than solely on the criteria that it is the disease source. More importantly, this approach allows the use of large-scale commercial culturing techniques for the production of antigens for infectious organisms which organisms are not generally amenable to such culturing techniques. Typically, the organisms which are not susceptible to culturation, in vitro, are those which cannot be sustained in presently available culture media, i.e. organisms which only propagate in a living host. Exemplary of such organisms are Ichthyophtirius multifiliis and Cryptocaryon irritans.

Ichthyophthiriasis, and its saltwater counterpart, produced by Cryptocaryon irritans, are epizootic diseases which claim high mortalities in aqua-culture. Ichthyophthirius multifiliis and Cryptocaryon irritans are holotrichous ciliates which are ectoparasitic on the gill and hypodermis of fish. The infective stage or tomite penetrates the epithelium where it encysts and develops into the parasitic stage. Macroscopically, these encysted forms produce the condition popularly known as "white spot disease" or "ick".

These parasites are known to cause severe epizootics in the culture of food fish, as well as ornamental fish. They are a major problem in high density ponds because the common chemical treatments are ineffective in removing the encysted stage. The encysted stage, after several maturation processes, divides into thousands of infective tomites, perpetuating the infection. Additionally, the common chemical treatments are also toxic to certain species and may be particularly dangerous in the treatment of young fish. The trend towards high density food fish production has increased the importance

of this disease.

The infective tomites of Ichthyophthirius multifiliis and Cryptocaryon irritans morphologically resemble the holotrichous ciliate, Tetrahymena pyriformis. Tetrahymena pyriformis, a carnivorous protozoan, is considered an opportunistic parasite of fish, rarely producing a primary disease. The close taxonomic association between these ciliates allows the use of antigenic cross-reactivity similar to the antigenic relatedness of canine distemper and measles virus.

The adult form of Tetrahymena pyriformis and the tomities of Ichthyophthirius multifiliis and Cryptocaryon irritans are similar in that they are all covered by cilia and that they are all pear (pyriform) shaped. The differences are that tomities of Ichthyophthirius multifiliis and Cryptocaryon irritans actively penetrate the skin of fish to form adult forms called trophozoites. Tetrahymena pyriformis does not induce classical "Ick" disease. Taxonomically, therefore, the three organisms are ciliates but belong to different genera.

The development of Tetrahymena pyriformis as an "heterotropic vaccine" for Ichthyophthirius multifiliis or Cryptocaryon irritans is efficacious for several reasons:

1) Tetrahymena pyriformis, a free living protozoan, is easily maintained in vitro using simple medium. Tetrahymena pyriformis is easily cultivated in the laboratory using a medium of water and almost any nitrogenous compound. Suitably, the medium is as simple as water with protein source and a carbohydrate source. A medium of serile water plus almost any other source of organic material found in a wide diversity ordinary bacteriological culture mediums is suitable, such as, sterile water plus 2-5% of sterile calf serum. A

particularly preferred medium is Elliot Medium #2 (Biology of Tetrahymena; Elliot, A.M.; Bowden, Hutchinson and Ross, Inc.; Stroudsburg, Pa. (1973)).

FORMULA FOR ELLIOT MEDIUM #2

Sodium Acetate $3H_2O$	1.0g
KH_2PO_4	1.0g
Proteose peptone	20.0g
Liver Extract "L"	1.0g
Glucose*	5.0g
Water	q.s. to 1000 ml

* glucose - filter, sterilize and add to medium after autoclaving

Cultivation takes place at a temperature in the range of about 0 to 35°C, preferably at about 26°C.

2) Tetrahymena pyriformis is naturally found in the same environment as Ichthyophthirius multifiliis.

3) Tetrahymena pyriformis, a relatively benign organism, can be used to protect against the pathogens Ichthyophthirius multifiliis and Cryptocaryon irritans based on the fact that selected antigens, i.e. cilia and mouth parts, are identical between these organisms.

A vaccine may be prepared by using the whole protozoan to produce a vaccine, i.e. disintegrating the whole protozoan to produce a vaccine. However, the cilia are readily removable and such use of the cilia is efficacious. The disintegration of the whole protozoan may be accomplished by techniques well-known in the art, e.g. grinding with a mortar and pestle, sonication, chemical treatment and enzyme treatment. The important point is that ciliary antigens protect the fish by producing an

immunity.

Preferentially, the vaccine comprise cilia stripped from Tetrahymena pyriformis according to the method of Rosenbaum (Rosenbaum, J.L. "Cilia Regeneration in Tetrahymena and Its Inhibition by Colchicine", J Cell Bio 40, p. 415 (1969)).

The antigenic material, i.e. the ciliary protein, may be separated from the whole cilia to more effectively concentrate the antigen.

Concentration may be achieved by methods known in the art, e.g., pervaporation, centrifugation and concentration by utlization of carbowax. Preferentially, the protein is concentrated with methyl cellulose and dialyzed against phosphate buffered saline (PBS) at 4°C. The protein concentration can then be assayed by the method of Lowry (Lowry et al, "Protein Measurement with the Follin Phenol Reagent", J Biol Chem., 193, p. 265 (1951)).

The vaccine additionally may comprise acceptable diluents, excipients or medicinal agents. Any substance which would not destroy or interfere with the antigenic material, i.e. ciliary protein, may be incorporated in the vaccine. Such substances include buffers, stabilizers, antibiotics, bacterial vaccines and nutritional supplements. Any acceptable diluent may be used, preferably, the vaccine is prepared in aqueous form. The vaccine may also include one or more adjuvants.

The vaccine may be administered by intraperitoneal injection, in capsule form, incorporated into food, and in the case of fish, by immersion of fish, or by spraying fish suspended in a net.

For mass aquacultural operations, immersion or spraying are preferred techniques. Such techniques are

well known for the administration of bacterial vaccines. Preferably, the vaccine is mixed with one or more bacterial vaccines when administered by immersion or spraying. Additionally, when administration is by the spray technique, it is preferable to include Bentonite in the spray formulation so as to enhance penetration and uptake of the vaccine.

The dose of antigen may be determined by the method of Hall (Hall et al, "Characterization Of A Teleost Immunogloublin: The Immune Macroglobulin From Channel Catfish", Comm. Biochem. Physiol. 46, pp. 187-197 (1973)).

In the case of a vaccine for Ichthyophthirius multifiliis or Cryptocaryon irritans derived from Tetrahymena pyriformis, the dosage rate is generally at least 2.5 micrograms of ciliary protein per 20 grams of fish. Preferably, the dose rate is about 5 micrograms of ciliary protein per 20 grams of fish.

The vaccine may be prepared as a concentrate and then diluted for use as needed. Lyophilization is a suitable method for the preparation of a concentrate. Alternatively, the vaccine may be stored at room temperature for short periods of time or may be kept frozen when long storage times are contemplated.

Vaccines prepared by the techniques set forth herein may suitably be applied to both lower and higher forms of life, e.g., fish, domestic animals and man. Typical examples include trout, salmon, catfish, carp, eel, tuna, bonito, dogs, cats, sheep cattle, horse and human.

Having essentially described this invention, a further understanding can be obtained by reference to certain specific examples which are provided herein for purposes of illustration only and are not intended to be limiting upon the scope of the invention.

EXAMPLE 1

Cultivation of T. pyriformis:

An axenic culture of T. pyriformis was obtained (Midwest Culture Service, Terre Haute, Ind.). Cultures were maintained in an enriched medium at pH 7.2 to 7.4 (Elliot Medium #2). All cultures were incubated aerobically at 26°C. At approximately three-week intervals fresh media was inoculated with 10% (v/v) of the old culture.

EXAMPLE 2

Cultivation of I. multifiliis:

I. multifiliis was maintained by serial passage in channel catfish (Ictalurus punctatus), which were kept in 38 liter (10 gallon) glass aquarium equipped with an undergravel filter. I. multifiliis was maintained in this manner for eight months.

EXAMPLE 3

Maintenance of Channel Catfish:

"Young of the Year" channel catfish, Ictalurus punctatus Rafinesque, were obtained from a commercial farm (J. Sims, Winder, Ga). Fish were kept in a 950 liter (250 gallon) flow-through stainless steel tank and were fed flake food (Kordon Corp., Hayward, California) supplemented with ground chicken liver on alternating days. For experimentation, fish were removed from the holding tank and allotted into 38 liter (10 gallon) glass aquaria equipped with undergravel filters. Aeration was provided by a central air supply attached to the air lift system of the gravel filter. Fish were sustained on the same diet schedule. Ammonia measured as NH_3-N and ni-

trate measure as NO_2-N were monitored throughout the experiments by standard test kits (LaMotte, Chestertown, Md.). Water was changed as necessary to avoid the accumulation of toxic products.

EXAMPLE 4

Preparation of T. pyriformis for Antigen:

Ten- to fourteen-day old cultures were used for antigen preparation. Organisms from 20 ml of culture were concentrated by centrifugation at 500 xG for 10 minutes (IEC Universal Model UV, International Equipment Co., Needham Hts., Mass.). The sediment was washed twice with phosphate buffered saline (PBS), resuspended in 2.5 ml of PBS and immediately used in the deciliation procedure. Deciliation was effected by a cold osmotic shock process followed by shearing through an 18 g needle (Rosenbaum et al). Deciliated cells were nonmobile. Ten ml of PBS was added to the tubes containing the deciliated cells, and the cells were concentrated by centrifugation for 10 minutes at 500 xG. The supernatant containing free ailia was decanted, placed in dialysis tubing, and concentrated with carboxymethyl cellulose. The concentrated cilia then were dialyzed overnight in PBS (pH 7.2) at 4°C. The deciliated cells were resuspended in PBS and stored at 4°C for later use as whole cell antigen. The protein concentrations of whole cell and ciliary antigen were determined (Lowry et al).

EXAMPLE 5

Preparation of I. multifiliis for Antigen:

Encysted trophozoites were removed from infected channel catfish by gentle scraping. The cysts were incubated at room temperature (20°C) in sterile distilled water containing 10 ml/l chloromycetin sodium succinate

(Parke-Davis, Detroit, Mich.) and 0.025 mg/l fungizone (GIBCO, Grand Island, N.Y.). After 18-24 hours, free swimming tomites were decanted, concentrated and deciliated in the same manner as described for T. pyriformis in Example 4.

EXAMPLE 6

In Vitro Testing:

Sera from fish and rabbits immunized with cilia and whole T. pyriformis cross-reacted wtih tomites of I. multifiliis. The antigenic relationship was demonstrated using three in vitro tests:

A. Immobilization Test -

In immobilization studies, live T. pyriformis exposed to rabbit antitetrahymena serum agglutinated at low dilutions and were immobilized at high dilutions. Tomites of I. multifiliis exposed to the same serum were immobilized within two hours. Titers in this heterologous test were 1:32 and 1:128. In contrast, immobilization titers in the homologous system were 1:1024.

B. Indirect Flourescent Antibody Test -

The treatment of both T. pyriformis and I. multifiliis with either rabbit anti-tetrahymena or rabbit anti-Ichthyophthirius sera lead to similar patterns of fluorescence when the organisms were stained with FITC labelled anti-rabbit IgG. With both organisms, the high-test staining was seen in the surface structures.

C. Passive Hemagglutination Test -

The passive hemagglutination test was used to provide a more sensitive indication of the cross-reactivity between I. multifiliis and T. pyriformis. In this test heterologous titers of 1:1024 were obtained (sheep erythrocytes tanned with Tetrahymena and rabbit anti-

Ichthyophthirius). These tests also showed cross-reactivity and indicate that an antigenic relationship exists between these two organisms, localized on ciliary antigens.

EXAMPLE 7

In Vivo Testing:

Twenty-five catfish, each weighing approximately 20 g, were allotted into 38 liter glass aquaria. Antigen was prepared as described in Example 4 and Example 5. The dosage of antigen per body weight of fish was determined according to Hall et al (Com. Biochem. Physiol. 46:187-197 (1973)). Fish were immunized by intraperitional injection of antigen diluted to working concentration with phosphate buffered saline (PBS). Each antigen dose was run in duplicate. Twenty-five non-immunized fish served as controls. The experimental groups were: (1) Tetrahymena cilia 5 µg; (2) deciliated Tetrahymena 10 µg; (3) Ichthyophthirius cilia 2.5 µg; and (4) deciliated Ichthyophthirius 2.5 µg. Higher concentration of tetrahymenid antigen were used because of the cross-reacting relation. Fifteen days after the initial immunization, booster injections were administered. Fish immunized with the tetrahymenid antigens received the same dose, while fish immunized with Ichthyophthirius cilia received 3.5 µg and deciliated Ichthyophthirius 4.5 µg, respectively. Fifteen days following the booster injections, an "Ich" infected contact fish was placed into each tank. The fish were observed for clinical signs of infection and mortality. The results are set forth in the following table:

TABLE I

Mortality of Channel Catfish Immunized with Ciliary and Whole Antigens of T. pyriformis, and I. multifiliis, and Challenged With I. multifiliis,* Two Weeks Following Secondary Immunization

Antigen	Primary Dose	Secondary Dose	Total # Fish	% Mortality
T. pyriformis cilia	5.0 µg	5.0 g	137	11.6
Whole (deciliated)	10.0 µg	10.0 g	89	100.0
I. multifiliis cilia	3.5 µg	3.5 g	120	42.5
Whole (deciliated)	2.5 µg	4.5 g	76	77.6
Nonimmunized			25	100.0

*Fish with clinical Ichthyophthiriasis were placed in each tank to expose experimental fish to disease.

Primary immunization of catfish with 5 µg of T. pyriformis ciliary antigen, followed by a secondary immunization with 5 µg of antigen two weeks later, conferred an excellent degree of protection against challenge with I. multifiliis when the fish were challenged two weeks after booster. Only 11.6% of the fish in these groups developed clinical disease. Fish immunized with 10 µg of deciliated whole cells, followed two weeks later with the same dose, were not protected against infection and died when exposed to disease. Homologous immunization with deciliated whole I. multifiliis cells (2.5 µg, 4.5 µg) resulted in 77.6% mortality following challenge with I. multifiliis. Mortality was 42.5% in fish immunized with homologous

ciliary antigen (3.5 µg, 3.5 µg).Mortality was 100% in the control groups.

Having now fully described this invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention set forth herein.

CLAIMS

1. A vaccine for protection against a pathogen comprising an antigen derived from a taxonomically similar organism.

2. The vaccine according to Claim 1, wherein the taxonomicaly similar organism has body parts identical to those body parts of the pathogen which exhibit antibody-antigen reactivity.

3. The vaccine according to Claim 2, wherein the body parts which exhibit antibody-antigen reactivity are determined by:

a. preparing a rabbit anti-pathogen serum;

b. contactng the rabbit anti-pathogen serum with the pathogen;

c. staining the so-contacted pathogen with FITC labelled anti-rabbit IgG: and

d. determining those body parts which exhibits the highest intensity of staining.

4. The vaccine according to Claim 1, wherein the pathogen is not readily cultured, in vitro.

5. The vaccine according to Claim 4, wherein the pathogen requires a living host for propagation.

6. The vaccine according to Claim 5, wherein the pathogen is an obligate parasite.

7. The vaccine according to Claim 5, wherein the obligate parasite is selected from the group consisting of Ichthyophthirius multifiliis and Cryptocaryon irritans.

8. The vaccine according to Claim 1, wherein said

taxonomiclly similar organism is readily cultured, in vitro.

9. The vaccine according to Claim 8, wherein said taxonomically similar organism is cultured using a culture medium of water and a nitrogenous compound.

10. The vaccine according to Claim 9, wherein said culture medium comprises water, a protein source and a carbohydrate source.

11. The vaccine according to Claim 9, wherein said culture medium comprises sterile water and sterile calf serum.

12. The vaccine according to Claim, wherein said culture medium is maintained at a T in the range of 0-35°C.

13. The vaccine according to Claim 1, wherein said taxonomically similar organism is an opportunistic parasite.

14. The vaccine according to Claim 13, wherein said opportunistic parasite is Tetrahymena pyriformis.

15. A vaccine for immunization of fish against obligate parasites derived from an opportunistic parasite.

16. The vaccine according to Claim 15, wherein said obligate parasite is selected from the group consisting of Ichthyophthirius multifiliis and Cryptocaryon irritans.

17. The vaccine according to Claim 15, wherein said opportunistic parasite is a ciliate.

18. The vaccine according to Claim 17, wherein said

opportunistic parasite is Tetrahymena pyriformis.

19. The vaccine according to Claim 15, wherein said obligate parasite is Ichthyophthirius multifiliis or Cryptocaryon irritans and said opportunistic parasite is Tetrahymena pyriformis.

20. A method of preparing a vaccine against a pathogen comprising:

a. innoculating a host animal with the pathogen;

b. collecting blood serum from the innoculated animal;

c. contacting the pathogen and the so-collected blood serum;

d. staining the so-contacted pathogen with fluorescent labelled anti-host animal IgG;

e. determining the surface features of the pathogen which exhibit the highest degree of staining;

f. cultivating an organism which has surface features identical to those of the pathogen which exhibited the highest degree of staining;

g. collecting said organism;

h. disintegratng said organism; and

i. recovering the proteinaceous material from said disintegrated organism.

21. An antigen preparation containing antigens from a parasite which is taxonomically similar to a parasite which is pathogenic to fish.

22. The antigen preparation according to Claim 21, wherein said preparation is a veterinary vaccine admin-

isterable by oral, parenteral, immersion or spray means.

23. An antigen preparation according to Claim 21, wherein said antigen is from Tetrahymena pyriformis.

24. A veterinary vaccine containing antigens from a parasite which is taxonomically similar to a parasite which is pathogenic to fish and an inert diluent.

25. The veterinary vaccine according to Claim 24, wherein said antigen is from Tetrahymena pyriformis.

26. The method of immunizing fish against infection by Ichthyophthirius multifiliis and Cryptocaryon irritans which comprises administering to the fish the vaccine of Claim 24.

27. The method according to Claim 25, wherein the vaccine is administered orally.

28. The method according to Claim 26, wherein the vaccine is administered by immersion.

29. The method according to Claim 26, wherein the vaccine is administered by spraying.

30. The method according to Claim 26, wherein the vaccine is administered by injection.

EDITORIAL NOTE

The applicant failed to renumber the amended claims in accordance with Section 205 of the Administrative Instructions.

Original claims 1-30 have been cancelled and, accordingly amended claims 1 to 6 are new.

AMENDED CLAIMS
(received by the International Bureau on 18 February 1981 (18.02.81))

1. A veterinary vaccine for immunizing fish against infection by Ichthyophthirius multifiliis and Cryptocaryon irritans comprising an effective amount of ciliary protein derived from Tetrahymena pyriformis cilia and an inert diluent.

2. A method of immunizing fish against infection by Ichthyophthirius multifiliis and Cryptocaryon irritans which comprises administering to the fish the vaccine of Claim 1.

3. The method according to Claim 2, wherein the vaccine is administered orally.

4. The method according to Claim 2, wherein the vaccine is administered by immersion.

5. The method according to Claim 2, wherein the vaccine is administered by spraying.

6. The method according to Claim 2, wherein the vaccine is administered by injection.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US80/01222

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. 3 A61K 35/68 A61K 39/002
U.S. Cl. 424/88

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/88

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstracts entry "TETRAHYMENA PYRIFORMIS"

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	U.S. A, 4009259 Published 22 February 1977 Ament et al	19
A	Chemical Abstracts 9th Coll. Index 1972-1976 Volume 76-85, pages 15516GS-15518GS Columbus, Ohio) entry "TETRAHYMENA PYRIFORMIS"	19
A	Beckert Dissertation Abstract Int. 36B, 11, 5461(1976) "Observations on the biology of ichthyophthirius multifiliis... and some immunological responses of channel catfish, ictalurus punctatus, to this parasite"	19
A	Hlond, Stefan Fao Fisheries Reports v.44(5): 365-368(1968) "Experiments in vitro culture of ichthyophth-irius multifiliis"	19
A	Beckert et al Proc. 18th Ann. Conf. S.E. Assoc. Game and Fish Comm. pages 438-441 (1964) "Some Host Response of Whate Catfish to Ichthyophthirius multifiliis, Fouquet"	19
A	Areerat, Thesis, Auburn Univ. Auburn, Ala. (1974) "The Immune Response of Channel Catfish, Ictalurus punctatus, (Rafinesque), to Ichthyophthirius Multifiliis" 37 pp.	19

* Special categories of cited documents: [15]

"A" document defining the general state of the art
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but on or after the priority date claimed
"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention
"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
12, December 1980	24 DEC 1980
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	Shep K. Rose

Form PCT/ISA/210 (second sheet) (October 1977)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter [12] not required to be searched by this Authority, namely:

1 to 18, 20 to 30

2. ☒ Claim numbers 1, because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [13], specifically:

the description and these claims fail to define with sufficient particularity the scope of the claim-encompassed indefinite terms such as: the operative species of antigen in claims 7, 16, 26, 29 to 30 which recite the pathogen to be protected against; or the pathogen to be protected against in claims 14, 18, 23, 25 and 27 which define the antigen. The rest of the claims define neither of the antigen or the pathogen, differing by being "taxonomically similar".

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.